Mitochondrial gene expression in small intestinal epithelial cells

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Most mitochondrial genes are transcribed as a single large transcript from the heavy strand of mitochondrial DNA, and are subsequently processed into the proximal mitochondrial (mt) 12 S and 16 S rRNAs, and the more distal tRNAs and mRNAs. We have shown that in intestinal epithelial biopsies the steady-state levels of mt 12 S and 16 S rRNA are an order of magnitude greater than those of mt mRNAs. Fractionation of rat small intestinal epithelial cells on the basis of their maturity has shown that the greatest ratios of 12 S mt rRNA/cytochrome *b* mt mRNA or 12 S mt rRNA/cytochrome oxidase I mt mRNA are found in the surface mature enterocytes, with a progressive decrease towards the crypt immature enteroblasts. Cytochrome *b* and cytochrome oxidase I mt mRNA levels are relatively uniform along the crypt–villus axis, but fractionation experiments showed increased levels in the crypt base. The levels of human mitochondrial transcription factor A are also greater in immature crypt enteroblasts compared with mature villus enterocytes. These results show that the relative levels of mt rRNA and mRNA are distinctly regulated in intestinal epithelial cells according to the crypt–villus position and differentiation status of the cells, and that there are higher mt mRNA and mt TFA levels in the crypts, consistent with increased transcriptional activity during mitochondrial biogenesis in the immature enteroblasts.

INTRODUCTION

The mitochondrial chromosome is a small circular molecule (16.5 kb in the human) which encodes mRNAs for some of the polypeptides of the mitochondrial respiratory chain [1,2]; these are translated within mitochondria themselves by special mitochondrial protein synthetic machinery (the mitochondrial chromosome also codes the 12 S and 16 S mitochondrial rRNAs and tRNAs). Most other mitochondrial proteins are encoded in the nucleus and synthesized on cytoplasmic ribosomes with an N-terminal signal sequence to target them for import into mitochondria [3,4].

Mitochondrial (mt) 12 S and 16 S ribosomal RNAs are transcribed as the proximal part of a long RNA precursor from the heavy strand of mt DNA [5–7]. Distal to the mt rRNAs, this also contains most of the mt mRNAs interspersed with tRNAs. The light strand of mt DNA is also transcribed into a RNA precursor; this contains the remaining few mRNAs and tRNAs.

While mutations and deletions of mt DNA have been well characterized in a number of neuromuscular diseases [8–12], the cellular expression of mitochondrial genes has received comparatively less attention. In cell culture systems, mt rRNAs are transcribed at a rate 15–60 times higher than mt mRNAs [5], and a protein factor which prematurely terminates H-strand transcription just distal to the 16 S mt rRNA has been identified (resulting in transcription of just the proximal 12 S and 16 S mt rRNAs and not the more distally located mRNAs and tRNAs) [13].

Relatively little is known about mitochondrial gene expression in differentiated cells. In rat hepatocytes and cerebellar cells, the ratio of mt mRNA to mt rRNA is an order of magnitude greater than that observed in HeLa cells [14,15]. Moreover, mt RNA levels decline relative to those of mt DNA during development of rat neural cells, and rise again in senescence [16].

Intestinal epithelial cells are derived from pluripotential stem cells near the base of the crypts, and differentiate as they migrate towards the luminal surface [17]. We have previously shown by *in situ* hybridization that the highest levels of 12 S and 16 S mt rRNA are found on the surface epithelial cells of the small intestine and colon, whereas mitochondrial respiratory chain activities have a relatively uniform distribution along the crypt–surface (villus) axis [18]. However, *in situ* hybridization experiments can suffer from problems of specificity and are at best semi-quantitative. In this paper we report studies of the relative levels of mt rRNAs and mRNAs in intestinal epithelial cells isolated from different levels along the crypt–villus axis in the small intestine.

MATERIALS AND METHODS

Isolation of intestinal RNA

Intestinal mucosal total RNA was isolated from four endoscopic biopsies obtained from the second part of the duodenum using an Olympus IT20 (wide channel) endoscope in six patients (three males and three females; mean age 42; range 26–68 years) and from the colon using an Olympus CF200HL colonoscope in six patients (two males and four females; mean age 48; range 31–66 years) in whom no endoscopic abnormality was detected. Two parallel biopsies from each patient were fixed in formalin and no abnormality was detected after independent histopathological assessment; furthermore, the results of other clinical workup, including biochemical and haematological profiles and upper abdominal ultrasound, were normal. A diagnosis of non-ulcer dyspepsia or irritable bowel syndrome was reached in each case.

Informed consent was obtained from all subjects, and the study was approved by the Ethical Committee of the Camberwell Health Authority.

Total RNA was prepared by an adaptation of the method of Chirgwin et al. [19], with the usual precautions to eliminate exogenous ribonuclease during processing [20]. The biopsy samples were homogenized with a Polytron in 3 ml of 5.2 M guanidium isothiocyanate, 0.5% (w/v) sodium lauryl sarcosine, 100 mM 2-mercaptoethanol and 25 mM sodium citrate (pH 7.0). RNA was separated by ultracentrifugation in a caesium chloride gradient as previously described [19].

Abbreviations used: mt RNA, mitochondrial RNA; h-mt TFA, human mitochondrial transcription factor A (= mt TF1); COX, cytochrome oxidase.

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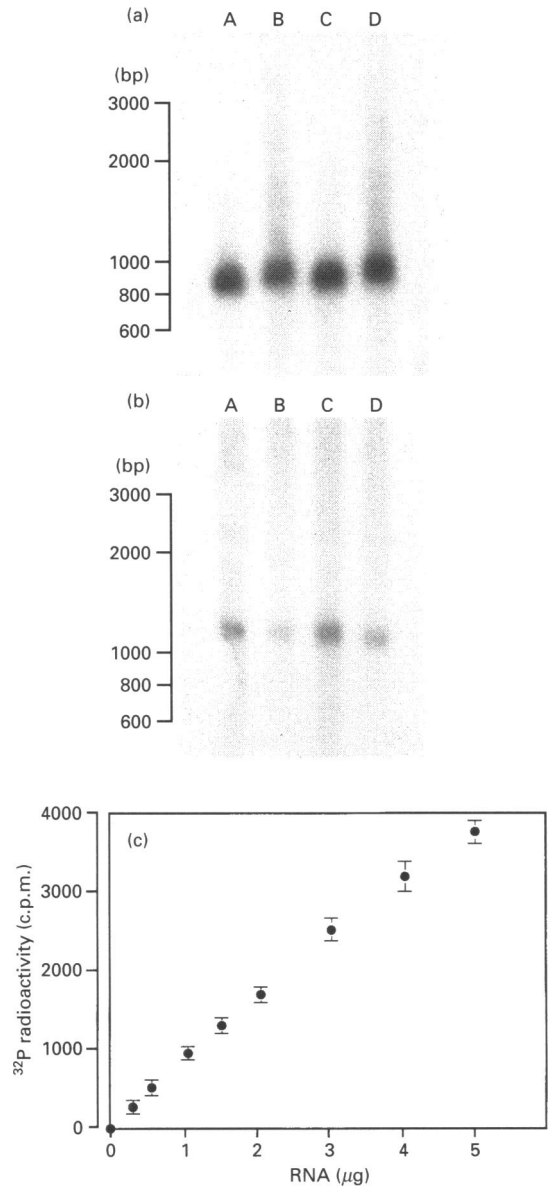


Figure 1 Northern hybridization of human intestinal RNA

Samples (15 μg) of human intestinal total RNA were separated by 1% glyoxal gel electrophoresis and transferred on to nitrocellulose. RNA ladder markers were run in one well and the corresponding strip of nitrocellulose was excised and stained separately. Four separate RNA preparations were run (lanes A–D). The filters wre hybridized with random hexamer primed ^{32}P-labelled probes to (**a**) 12 S mt RNA, and (**b**) cytochrome *b* mt mRNA. (**c**) Slot-blot hybridization of 12 S mt RNA and cDNA showing linearity of hybridization response. RNA (0–8 μg) was applied in triplicate to nitrocelluluose using a slot-blot apparatus and the filter was dried and hybridized with a random hexamer primed 12 S cDNA ^{32}P-labelled probe. The bands were excised and quantified by β-counting.

Northern hybridization

Samples of RNA (15 μg) were denatured by treatment with glyoxal and dimethyl sulphoxide as described by McMaster and Carmichael [21]. These were loaded in 50% glycerol, 10 mM sodium phosphate (pH 7.0) and 0.4% Bromophenol Blue on to a 1.2% agarose gel in 10 mM sodium phosphate (pH 7.0) buffer. RNA ladder markers were loaded in a lane on the edge of the gel, which was run at 3 V/cm in a submarine electrophoretic system with recirculation of the buffer from the cathode to the anode. After completion of electrophoresis, the RNA samples and markers were transferred to Hybond-N (Amersham) by capillary blotting. The filters were dried at room temperature and baked at 80 °C for 2 h before prehybridization for 2–6 h at 65 °C in 6 × SSC, 5 × Denhardt's, 0.1% SDS and 100 μg/ml denatured sheared salmon sperm DNA. Hybridization was at 65 °C for 18 h in prehybridization buffer with a ^{32}P-labelled random hexamer primed probe [22,23]. Unbound radioactivity was removed by two 15 min washes in 2 × SSC/0.1% SDS, the first at room temperature and the second at 65 °C. A final 15 min wash in 0.2 × SSC/0.1% SDS was carried out at 65 °C. Hybridization was detected by autoradiography using Hyperfilm MP (Amersham) at −70 °C.

Fractionation of rat intestinal epithelial cells

Intestinal epithelial cells were isolated from different positions along the crypt–villus axis by a modification of the method of Weiser [24]. Segments of jejunum (15 cm) distal to the ampulla of Vater were freshly removed from anaesthetized Wistar rats, these were tied at each end, and the sac was filled with 1.5 mM KCl, 96 mM NaCl, 27 mM sodium citrate, 8 mM KH_2PO_4 and 5.6 mM Na_2HPO_4 (pH 7.3), and incubated at 37 °C in Hanks' buffered saline solution (HBSS) for 15 min. This buffer was then removed and the sac was refilled with 2.6 mM KCl, 137 mM NaCl, 1.47 mM KH_2PO_4, 8 mM Na_2HPO_4, 1.5 mM EDTA and 0.5 mM dithiothreitol (pH 7.3) and incubated at 37 °C in HBSS for successive periods of 4, 5, 5, 7, 12 and 20 min. At the end of each period the solution containing the enterocyte fraction was removed from the sac and replaced with fresh solution for the next fraction. Total RNA was prepared from each fraction as described above, and a sonicated portion as used to assay γ-glutamyltransferase activity and protein content.

Slot-blot hybridization

RNA (1 μg or 4 μg) was denatured by heating to 65 °C for 10 min in 200 μl of 7.5% formaldehyde/6 × SSC. A series of different RNA samples corresponding to each intestinal enterocyte fraction was applied in triplicate to a nitrocellulose filter using a Schleicher & Schuell manifold. Two further replicate filters for fractionated enterocyte experiments or eight replicate filters for unfractionated intestinal RNA experiments were made at the same time, with identical loading and orientation of the RNA samples. Every filter also had duplicate or triplicate samples of unfractionated intestinal RNA (0–8 μg) to confirm linearity of the hybridization response with RNA concentration. Each filter was dried, baked and hybridized as described for Northern hybridization above. Probe details are given in the legend to Table 1. Hybridization was quantified by β-counting the bands excised from each filter.

Protein assay

Protein was assayed using the bicinchoninic acid (BCA) method (Pierce) [25].

γ-Glutamyltransferase (EC 2.3.2.2.) activity

γ-Glutamyltransferase activity was assayed [26,27] in 100 μl samples of diluted (1:10–1:200), sonicated (four bursts of 15 s; 15 μm peak-to-peak; Soniprep 150), enterocyte fractions. Each 0.1 ml sample was incubated for 30 min at 37 °C with 250 μl of sonicated 0.2 mM γ-glutamyl-7-amino-4-methylcoumarin in 0.1 M Ammediol buffer, pH 8.5, containing 20 mM glycylglycine and 0.1% Triton X-100. The reaction was terminated in 2 ml of

Table 1 Levels of expression of mt RNA species in mucosal biopsies of human colon and small intestine

A series of identical slot-blot filters (one for each probe used) was prepared with triplicate 1 μg and 4 μg samples of RNA from six different RNA preparations from human mucosal biopsies of the small intestine or colon. Each filter was probed separately with a random hexamer primed probe for each RNA species (with uniform specific activity), and bound probe was quantified by radioactive counting of excised bands. Results are expressed as a ratio to mitochondrial cytochrome *b* mRNA levels, and are means ± S.D. ($n = 6$). Each probe gave a single band with human intestinal RNA on a Northern blot of the correct size, as shown in the final column. Probes were: cDNA to 12 S mt rRNA (bases 1057–1505, [1]); 16 S mt rRNA [37]; 18 S rRNA [38]; cytochrome *b* (bases 14807–15232, [1]); NADH dehydrogenase hIV (bases 11632–11862, [1]); COX I (bases 5953–6585, [1]); COX VIIc [39] and ATPase VI (bases 8663–9086, [1]).

Probe	Levels of expression		mRNA size on Northern blot (bp) [RNA sequence bp]
	Small intestine	Colon	
12 S mt rRNA	12.4 ± 2.8	11.5 ± 1.8	960 [954]
16 S mt rRNA	15.1 ± 3.6	14.6 ± 2.1	1480 [1560]
18 S rRNA	412 ± 91	314 ± 82	1860 [1869]
Cytochrome *b* mt mRNA	1.0 ± 0.3	1.0 ± 0.4	1130 [1145]
NADH dehydrogenase h (IV) mt mRNA	1.1 ± 0.5	0.8 ± 0.2	1780 [1667]
COX I mt mRNA	1.2 ± 0.4	1.7 ± 0.8	1660 [1542]
COX VIIc cytoplasmic mRNA	0.9 ± 0.2	1.1 ± 0.8	2120 [only partial sequence known]
ATPase VI mt mRNA	1.3 ± 0.3	1.2 ± 0.5	880 [678]

ice-cold 50 mM glycine/NaOH buffer (pH 10.4), and samples were kept on ice for assay of 7-amino-4-methylcoumarin levels by spectrofluorimetry (excitation 370 nm, emission 400 nm). A standard curve of 0–20 μM 7-amino-4-methylcoumarin was established using the commercially purified (Aldrich) authentic compound, sonicated prior to use. Results were expressed as activity (nmol/min per mg of protein).

Measurement of human mitochondrial transcription factor A (h-mt TFA) along the small intestinal crypt–villus axis

Intestinal epithelial cells were obtained according to the method of Traber et al. [28] from a 15 cm segment of mid-ileum obtained at surgery from a patient undergoing a small bowel resection for a tumour distal to the specimen. The intestine was otherwise histologically normal and not distended at the time of surgery. The lumen was immediately rinsed with ice-cold 0.15 M NaCl containing 40 μg/ml PMSF. The intestinal segment was tied at each end and filled with 96 mM NaCl, 27 mM sodium citrate, 1.5 mM KCl, 8 mM KH_2PO_2, 5.6 mM Na_2HPO_4 and 40 μg/ml PMSF (pH 7.3) preoxygenated with 100% O_2. This was incubated for 15 min at 37 °C in a bath of oxygenated 0.15 M NaCl. Following this the buffer was removed and discarded, and the segment was filled with oxygenated 109 mM NaCl, 2.4 mM KCl, 1.5 mM KH_2PO_4, 4.3 mM Na_2HPO_4, 1.5 mM EDTA, 10 mM glucose, 5 mM glutamine, 0.5 mM dithiothreitol and 40 μg/ml PMSF (buffer B) and incubated in the 0.15 M NaCl bath at 37 °C for a further 10 min. This solution was then removed and the cells were pelleted, washed twice in buffer B and homogenized in PBS [29] using a Dounce homogenizer before being freeze–thawed three times in liquid nitrogen. The segment was refilled with buffer B and further fractions were successively taken as described above by incubation for 10, 10, 10, 20, 20, 30 and 30 min.

To quantify h-mt TFA, an antibody-capture ELISA [29] was used with affinity-purified polyclonal rabbit anti-(h-mt TFA) that specifically detects the 25 kDa h-mt TFA protein on Western blots [30]. Microtitre plates (96 wells; Maxisort; Nunc) were coated with 50 μl of the different enterocyte fractions in freshly prepared carbonate buffer [15 mM Na_2CO_3, 35 mM $NaHCO_3$, 0.02% (w/v) sodium azide] in triplicate at a protein concentration of 50, 10, 5, 1, 0.5 and 0.1 μg/ml by incubation at 4 °C overnight. For each enterocyte fraction a second set of triplicate wells was coated at each protein concentration to serve as blanks (without the addition of antiserum). The plates were each washed three times with PBS containing 0.05% (w/v) Tween 20 [29] before blocking with 10% (v/v) pre-immune rabbit serum in PBS for 2 h at 25 °C and washing a further three times in PBS-Tween. Affinity-purified anti-(h-mt TFA) antiserum [30] (1:2000 dilution) was then added to each of the triplicate wells, with PBS-Tween being added to the blank wells. After incubation for 2 h at 25 °C the plates were again washed in PBS-Tween, and 50 μl of 1:150 diluted biotinylated anti-(rabbit antibody) (PK4001; Vector Laboratories) was added to each well and incubated for a further 2 h at 25 °C. The plates were again washed three times with PBS-Tween, and each well was incubated with avidin–(horseradish peroxidase) complex (Vector Laboratories), and the bound peroxidase enzyme was detected by the addition of 50 μl 4 mg/ml *o*-phenylenediamine hydrochloride in 50 mM phosphate citrate buffer (pH 5.0) and 0.03% (w/v) sodium perborate. This reaction was stopped after 6 min by the addition of 50 μl of 3 M sulphuric acid, and the absorbance in each well was read at 495 nm using a Titertek MCC/340 plate reader.

The use of excess anti-(h-mt TFA) antibody and second antibody was investigated in preliminary experiments with serial antibody dilutions on saturating amounts of antigen (50 μl of 100 μg/ml). Immunoblots were also carried out to confirm that a single band of 25 kDa h-mt TFA was seen in the intestinal enterocytes. For each sample a curve of A_{495} against protein concentration was plotted after subtraction of blanks. The relative amounts of h-mt TFA in each sample in relation to the enterocyte fraction at the villus tip were determined from the protein concentrations at the linear mid-point ($A_{495} = 0.8$) of the curves.

RESULTS

Mitochondrial RNA levels in endoscopic biopsies of small intestinal mucosa

To show that the correct mt RNA species were being detected in these experiments, RNA from intestinal biopsies was separated by glyoxal gel electrophoresis for Northern hybridization. The nitrocellulose filters were separately probed for different mt RNA species (Figure 1 and Table 1). In each case a single band

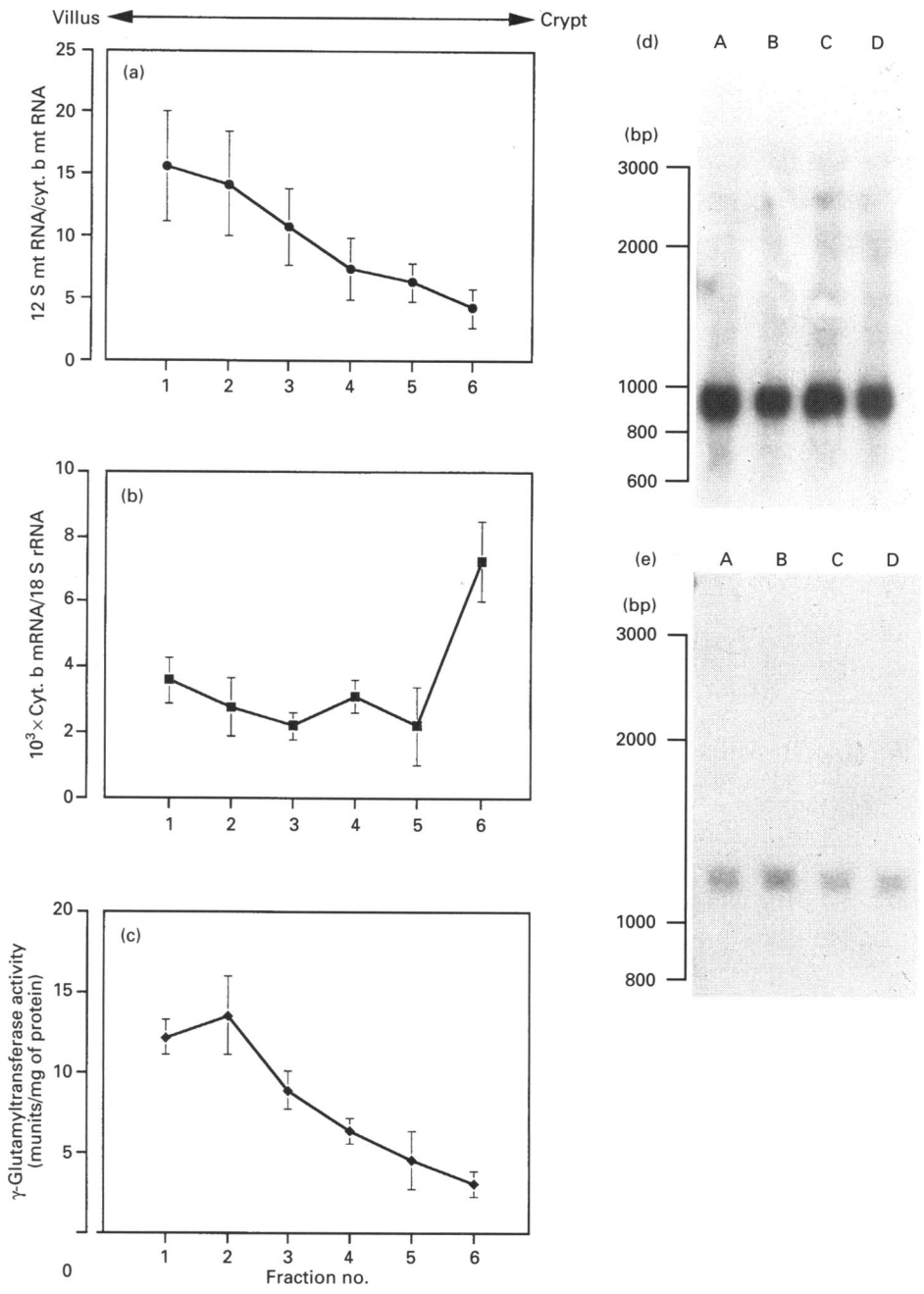
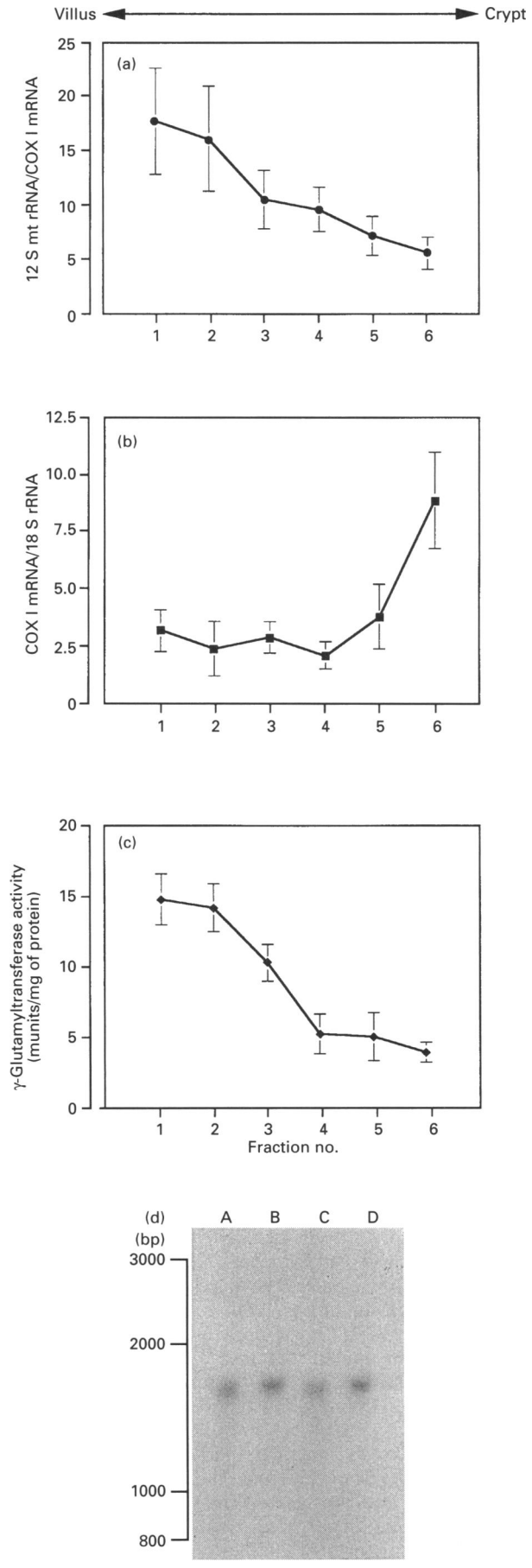


Figure 2 Measurement of 12 S mt rRNA and cytochrome *b* mt mRNA levels in rat small intestinal epithelial cells isolated in different fractions along the crypt–villus axis

Fractions of small intestinal epithelial cells were isolated from different positions along the crypt–villus axis of the proximal rat small intestine. RNA preparations from these samples were assayed in triplicate by slot-blot hybridization to determine the ratios (means ± S.D.) of (**a**) 12 S mt rRNA/cytochrome *b* (cyt. *b*) mt mRNA, and (**b**) cytochrome *b* mRNA/18 S cytoplasmic rRNA. Control slots of unfractionated rat intestinal RNA (0–8 μg) were used to verify that the slot hybridization was in the linear range for both probes. Samples of each fraction were assayed in triplicate (means ± S.D.) for γ-glutamyltransferase activity to verify that a gradient had been obtained (**c**). Four separate fraction experiments were carried out with identical results. Northern hybridization of four separate unfractionated samples (lanes A–D) of rat small intestinal RNA was carried out to confirm that probes were detecting a unique species of the correct predicted size: (**d**) 12 S mt rRNA; (**e**) cytochrome *b* mt mRNA.

was observed, with the calculated size of RNA in good agreement with the published sequences (Table 1).

The relative levels of the mt RNA species were estimated by slot-blot hybridization. Eight identical filters were prepared containing triplicate 1 μg samples of six endoscopic biopsy RNA preparations, and each filter was separately hybridized with a probe for the eight different mt RNA species tested. Care was taken to ensure that the probes were of uniform size and specific radioactivity. Linearity of the hybridization responses in this range was confirmed for each probe by serial dilutions of RNA in control slots (shown for 12 S mt rRNA, in Figure 1c). This also showed that the experimental system was satisfactorily in



probe excess. Moreover, no hybridization was seen with mt cDNA sequences in control slots other than with the correct target sequence. Results of β-counting of excised bands were expressed in terms of the ratio to cytochrome *b* mRNA hybridization.

Mt 12 S and 16 S rRNAs were shown to be expressed at higher steady-state levels than mt mRNAs in both small intestinal and colonic biopsies, with ratios to cytochrome *b* mt mRNA of between 11 and 16 (Table 1). The steady-state levels of mitochondrial-encoded NADH dehydrogenase IV, cytochrome oxidase I (COX I) and ATPase VI, and of the nuclear-encoded COX VIIc and NADH dehydrogenase VI, were not significantly different from mitochondrial cytochrome *b* mRNA levels (Table 1).

Mitochondrial rRNA and mRNA levels in rat small intestine epithelial cell fractions along the crypt–villus axis

The levels of mt 12 S rRNA and cytochrome *b* mRNA were examined in epithelial cell fractions isolated from freshly obtained rat small intestinal sacs (Figure 2). Northern hybridization with rat intestinal RNA confirmed that a single band of the correct size was being detected by both the 12 S mt rRNA and the cytochrome *b* mt mRNA probes (Figures 2d and 2e). Results showed that there was a progressive increase in the 12 S mt rRNA/cytochrome *b* mt mRNA ratio, from 4 at the base of the crypts to 16 at the villus surface. Assay of γ-glutamyltransferase activity in the fractions confirmed that a gradient was obtained, consistent with successful villus–crypt fractionation as previously reported [24].

We also found that cytochrome *b* mt mRNA showed relatively uniform levels along the length of the crypt–villus axis, except that there were significantly higher levels (consistently in four separate fractionation experiments) in the last crypt fraction (with the results expressed as a ratio to 18 S rRNA levels). To confirm this result for a different mt mRNA, we carried out the experiment with 12 S rRNA and COX mRNA. Again, the 12 S rRNA/COX I mRNA ratio increased from 5 to 15 along the crypt–villus axis, although the highest levels of COX I were seen in the crypt fraction, with relatively uniform levels elsewhere along the axis (Figures 3a–3c). Northern hybridization with rat intestinal RNA also confirmed that the COX I probe detected a band of the correct size (Figure 3d). Very similar results were also seen with NADH dehydrogenase IV mt mRNA (not shown).

Levels of h-mt TFA in human small intestinal epithelial cells along the crypt–villus axis

To assess the levels of h-mt TFA in the small intestine we used enterocyte fractions obtained at different levels of the villus–crypt axis of human ileum. Rat tissue was unsuitable for this experiment since bands other than the 25 kDa mt TFA were detected in Western blots using the anti(h-mt TFA) antiserum. Conversely, the increased ischaemic time inherent in human intestinal resections during mesenteric vessel ligation results in reduced yields

Figure 3 Measurement of 12 S mt rRNA and COX I mRNa levels in rat small intestinal epithelial cells isolated in different fractions along the crypt–villus axis

Details are as described in the legend to Figure 2. (**a**) Ratio of 12 S rRNA/COX I mt mRNA, (**b**) COX I mt mRNA/18 S cytoplasmic rRNA, (**c**) γ-glutamyltransferase activity. (**d**) Northern hybridization of separate unfractionated samples (A–D) of rat small intestinal RNA was carried out to confirm that the COX I probe was detecting a unique species of the correct predicted size.

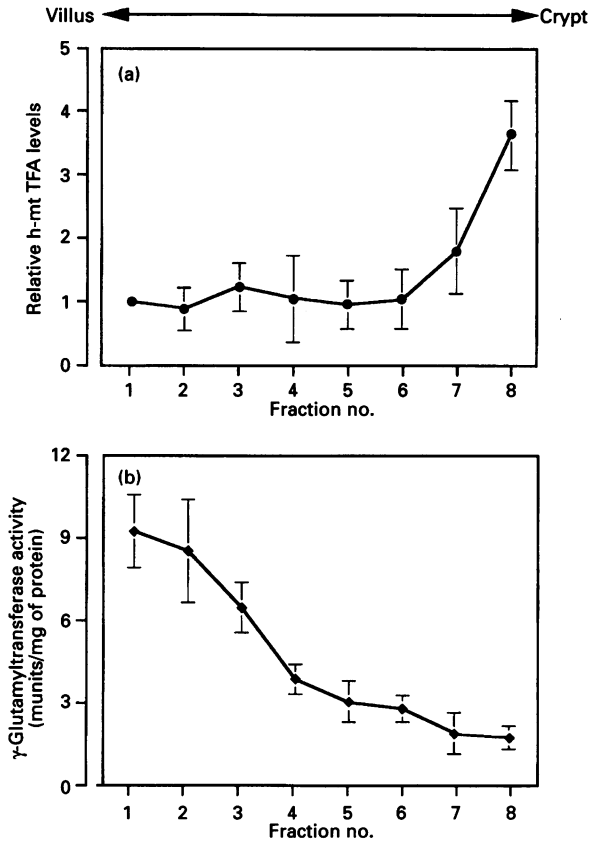


Figure 4 Relative levels of h-mt TFA in human small intestinal epithelial cells isolated in different fractions along the crypt–villus axis

Fractions of human small intestinal epithelial cells were isolated from different positions along the crypt–villus axis. The cells were homogenized and freeze–thawed, and loaded in triplicate on to microtitre plates at serial protein dilutions. The relative levels of h-mt TFA (means ± S.D.) were determined with respect to the villus surface fraction by antibody capture ELISA using affinity-purified anti(h-mt TFA) antibodies.

of RNA from such tissue [31], so rat enterocytes were more satisfactory for the mitochondrial gene expression experiments.

ELISA of villus–crypt small intestinal enterocyte fractions for h-mt TFA showed uniform levels, apart from in the two distal crypt fractions, where significantly increased levels were seen (Figure 4). Serial dilutions of small intestinal proteins on the ELISA confirmed that readings were being taken in the linear range, and Western blots (not shown) showed binding to a single protein band of 25 kDa, as previously described for mt TFA [30,32].

DISCUSSION

By fractionation of small intestinal epithelial cells along the crypt–villus axis we have shown that the ratio of 12 S mt rRNA/cytochrome *b* mt mRNA (or 12 S mt rRNA/COX I mt mRNA) decreases from 16 to 4 from the surface progressively towards the crypt base. Since the levels of mt mRNAs are constant for most of the crypt–villus axis, the change in ratio is largely accounted for by a progressive decrease in mt rRNA levels from the villus surface into the crypts. However, the mt rRNA/mt mRNA ratio does not fall significantly when mt mRNA levels are increased at the crypt base, as there is also a small increase in mt rRNA levels in the basal enterocytes compared with those in the mid-crypt, although these basal mt rRNA levels are less than those at the villus surface. Our previous *in situ* hybridization experiments showed high levels of 12 S and 16 S mt rRNAs on the surface epithelial cells of the human small intestine and colon, with lower levels down the surface–crypt axis, but these results were not quantitative [18]. Mitochondrial respiratory chain enzyme activities (and hence mitochondrial content) were shown to be relatively uniform along the surface–crypt axis [18].

Since the intestinal epithelial cells arise from pluripotential cells near to the base of the crypt and the differentiated cells migrate upwards to be shed from the villus tip after 4 days [17], the changes in mt rRNA/mRNA ratios represent different stages in the enterocyte life-cycle.

It is clear that the levels of mt rRNAs at all stages are greater than the levels of mt mRNAs or nuclear-encoded mRNAs for mitochondrial proteins. Kruse et al. [13] have characterized a protein (mTERF), present at very low abundance [33], which terminates heavy-strand transcription distal to the mt rRNA genes and proximal to the heavy-strand mt mRNA genes, so there is a relatively higher rate of heavy-strand mt rRNA synthesis. While it is possible that increased activity of mTERF may contribute to the increasing mt rRNA/mt mRNA ratios along the crypt–villus axis, this would also require an increased heavy-strand transcriptional rate, as the mt mRNA levels remain constant. Moreover, levels of h-mt TFA are constant from the mid-crypt towards the surface, although it is very likely that there are other factors which also contribute to the regulation of mitochondrial transcription [34]. Another possibility is that the increasing mt rRNA/mt mRNA ratios along the crypt–villus axis are explained by differences in mt rRNA and mRNA degradation rates. In HeLa cells the $t_{\frac{1}{2}}$s of mt rRNAs have been estimated at 2.5–3.5 h, and those of mt mRNAs between 25 and 90 min [35], which is comparatively short in relation to the crypt–villus enterocyte migration time of 4 days. Because of the limited viability of extracted differentiated enterocytes, we have not been able to study directly the kinetics of synthesis and degradation of mt rRNAs and mRNAs, or whether there is increased stability of mt rRNAs in the surface villus enterocytes. Thus we are not able to distinguish between transcriptional and degradation mechanisms to explain the increase in the mt rRNA/mRNA ratios along the crypt–villus axis.

We have shown that mt mRNA levels are relatively constant (compared with those of 18 S rRNA itself constant along the crypt–villus axis [18,36]) from the villus tip towards the crypt, apart from the enterocyte fraction in the base of the crypt where the levels are significantly higher. The constant levels of mRNAs throughout the villus enterocytes are consistent with the requirement to maintain high levels of mitochondrial enzymes [18] in view of the energetic requirements of transport and absorptive functions in these cells. In contrast, higher levels of mt mRNA are seen in the immature cells of the crypt base; this is consistent with the requirements of mitochondrial biogenesis. Increased levels of mt mRNAs have also been found in developing cerebellar cells compared with adult cells [15].

h-mt TFA binds to a site upstream of the heavy-strand promoter and to another site upstream of the light-strand promoter [37], and has been shown to have a concentration-dependent effect on transcription initiation using *in vitro* systems [30,34]. The heavy-strand RNA precursor is subsequently processed into mt rRNAs, mt mRNAs and mt tRNAs. We have found increased levels of this h-mt TFA in the intestinal crypt bases, suggesting that the increased levels of cytochrome *b* and COX I mRNAs (and mt rRNAs) result from increased heavy-strand transcriptional activity in these immature crypt cells.

These studies on the intestinal epithelium show that the expression of the mitochondrial genome alters during cellular maturation in the intestinal epithelium as the enterocytes migrate towards the villus surface. The highest mt mRNA levels at the crypt base may be consistent with increased transcriptional activity during mitochondrial biogenesis, whereas the relative increase in mitochondrial rRNAs in the surface enterocytes may reflect one of the senescent changes prior to extrusion from the surface. The system should form a useful model in the understanding of the regulation of mitochondrial gene expression in developing and senescent cells.

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